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GENETICS AND MOLECULAR BIOLOGY OF PLASTIDS OF HIGHER PLANTS

(plastids in male gametes, mutation induction, nitroso-urea, plastid mutants, thylakoid proteins, plastid ribosome deficiency, pollen, chloroplast, photosynthesis, mitochondria)

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SUMMARY

In the majority of angiospermous species the plastids are excluded from the generative or sperm cells during the first pollen mitosis or during pollen development; this is the basis of the maternal inheritance of plastid genes. In a smaller group of species there is, however, an equal distribution of plastids during the first pollen mitosis. Consequently generative and sperm cells of these species contain numerous plastids, and there is biparental plastid inheritance .

N-nitroso-N-methyl-urea and N-nitroso-N-ethyl-urea are chemicals potent for the induction of plastome mutations in higher plants (Antirrhinum, Lycopersicon, Helianthus, Saintpaulia).

Our research group analyzed three mutants of Antirrhinum and Pelargonium: they proved to have specific defects in photosystem I or photosystem II and an associated loss (or marked decrease) in specific thylakoid proteins which are connected with the photosystems.

In Pelargonium and Hordeum we found several plastome mutants which have no (or almost no) plastid ribosomes, but normal amounts of cytoplasmic ribosomes. These mutants are very suitable tools for the study of the synthetic capacity of the plastidal protein synthesizing system. Using these mutants a number of plastid components have been characterized which are synthesized on cytoplasmic ribosomes and then transported into the plastids. On the other hand, four protein complexes have been proved to be missing in the mutant deficient plastid ribosomes, because some of their polypeptides are normally synthesized on plastid ribosomes and are consequently not formed in these mutants. These are ribulose-1,5-biphosphate carboxylase/oxygenase, plastidal coupling factor CF_1, and the protein complexes for photosystem I and II.

INTRODUCTION

The participation in the Stadler Genetics Symposium gives me satisfaction, because - from the viewpoint of the history of genetics - I feel a kind of scientific kinship with Lewis Stadler and the Department in which he worked. While a postgraduate student I worked with the German plant geneticist Hans Stubbe. At the time of the International Congress of Genetics in Berlin in 1927 Stubbe was a young coworker of Erwin Baur, the president of that Congress. After Herman J. Muller's lecture on the induction of mutations in Drosophila by X-rays and after the publication of Stadler's work on mutagenesis in barley and maize, Baur asked Stubbe to start the same type of experiments in Germany with the ornamental plant *Antirrhinum majus*. For the following decade Stubbe became the leading geneticist for mutation induction in plants in Germany and studied similar problems as Stadler did in the United States. Thus, in a figurative sense I would regard Stadler as my scientific uncle.

My present talk deals with the work on plastid genetics carried on in the Department of Genetics of the Faculty of Natural Sciences of the Martin-Luther-University in Halle. Our Department is relatively young. It was founded only in 1967 and has become the center for the specialization of biology students in genetics in the German Democratic Republic. I am concerned with three problems which my coworkers and I have been studying during the past year; these are:
(i) the content of plasmatic organelles, especially plastids and mitochondria, in the pollen grains and tubes of higher plants,
(ii) the experimental induction of plastid mutations by nitrosourea-compounds, and
(iii) the analysis of plastid mutants as a tool for unravelling the coding capacity of the plastid DNA and for defining the synthetic abilities of the plastid ribosomes.

ORGANELLE CONTENT OF MALE GAMETOPHYTES AND GAMETES OF HIGHER PLANTS

One starting point in our studies was the fact that angiosperms form two groups regarding the inheritance of plastids: In the majority of species there is a uniparental, purely maternal inheritance of plastid characters, e.g. in Hordeum, Zea, Beta, Nicotiana, Petunia, Mirabilis. In contrast, in a minority of species a clear biparental inheritance of plastid differences if found, e.g. in Pelargonium, Oenothera and Hypericum. It is obvious that the reason for these differences can only be found by cytological, electron microscopical studies of pollen development, the fertilization process, zygote formation and embryogenesis. This led us to begin such studies in our Department carried out mainly by Dr. Knoth and Mrs. Krahnert.

In all angiosperms the microspores, formed by meiosis, contain numerous plastids, mitochondria, dictyosomes etc. The first pollen mitosis leads to the formation of a vegetative cell (which later grows out into the pollen tube) and a generative cell. The generative cell is in most species initially attached

TABLE 1. Plastid content in male gametophytes and gametes of angiosperms: Type 1 (P+: plastids present, P-: plastids absent).

Species	veg. cell	gen. cell	sperm cell	Reference
Gossypium hirsutum	P+	P-	P-	JENSEN, FISHER '68, '70 JENSEN '72
Hordeum vulgare	P+	P-	P-	CASS, KARAS '75
Beta vulgaris	P+	P-	P-	HOEFERT '69
Agropyrum repens	P+	P-		LOMBARDO, GEROLA '68b
Ambrosia psilostachya	P+	P-		LARSON '65
Antirrhinum majus	P+	P-		KNOTH '76
Bellevalia lipskyi	P+	P-		KORDYUM et al. '75
Capsella bursa-pastoris	P+	P-		SCHULZ, JENSEN '68
Carya pecan	P+	P-		LARSON '65
Castilleia foliosa	P+	P-		JENSEN, ASHTON, HECKARD '74
Endymion non-scriptus	P+	P-		ANGOLD '68
Epidendrum scutella	P+	P-		COCUCCI, JENSEN '69
Impatiens balsamina	P+	P-		DUPUIS '74
Impatiens walleriana	P+	P-		VAN WENT '74
Lycopersicon esculentum	P+	P-		KRAHNERT '79 unpubl.
Lycopersicon peruvianum	P+	P-		CRESTI et al. '75
Mirabilis jalapa	P+	P-		LOMBARDO, GEROLA '68a
Muscari racemosum	P+	P-		KORDYUM et al. '75
Parkinsonia aculeata	P+	P-		LARSON '65
Petunia hybrida	P+	P-		SASSEN '64, VAN WENT '70
Quercus virginiana	P+	P-		LARSON '65
Tradescantia paludosa	P+	P-		KAUFMANN et al. '62, MARUYAMA et al. '65, MARUYAMA '68
Triticum aestivum	P+	P-		KNOTH '79 unpubl.
Zea mays	P+	P-		LARSON '65

to the cell wall of the microspore; later on it becomes a spindle-shaped cell completely surrounded by the vegetative cell.

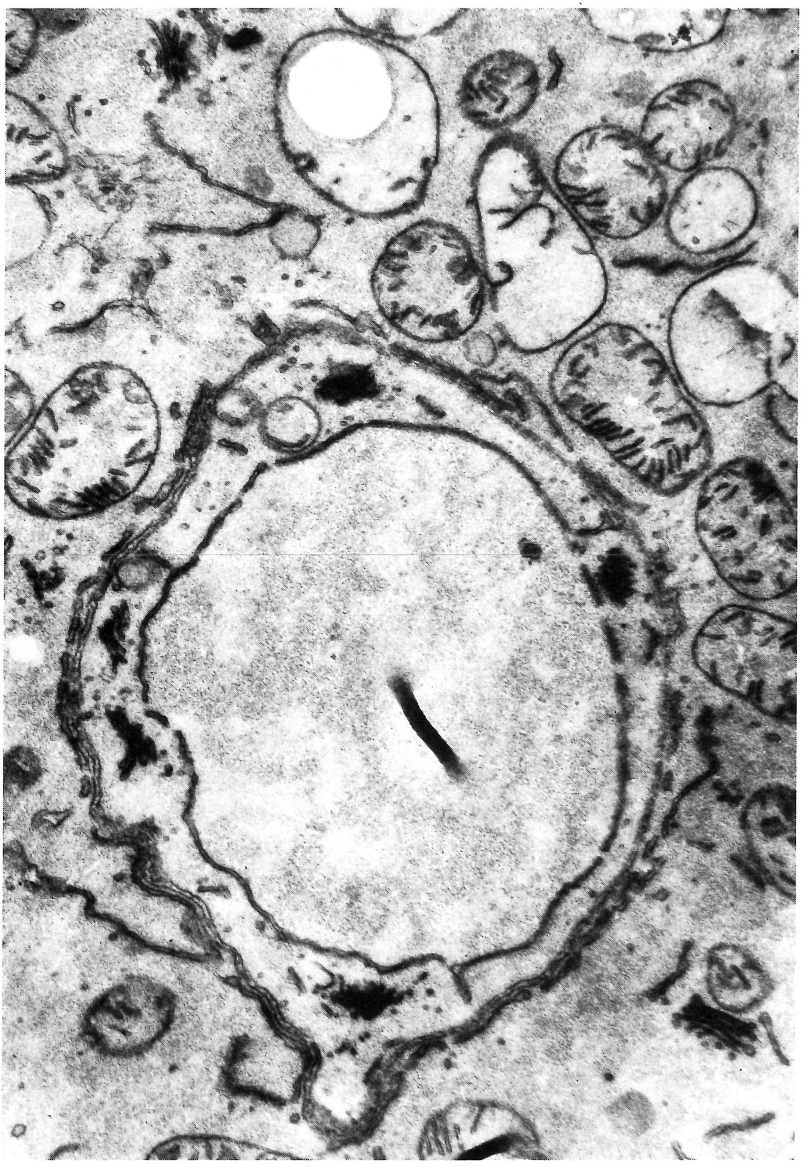

FIGURE 1. Electron micrograph of pollen cells of *Lycopersicon esculentum*. The generative cell with a big generative nucleus contains some mitochondria and dictyosomes, but has no plastids. In the vegetative cell there are many plastids with starch grains, many mitochondria and other plasmatic constituents. Fixative: potassium permanganate.
(Electron micrograph: S. Krahnert)

TABLE 2. Plastid content in male gametophytes and gametes of angiosperms: Type 2

Species	veg. cell	gen. cell	sperm cell	cross	Reference
Solanum chacoense tuberosum	P+	P+ young	P-		CLAUHS, GRUN '77
Hyoscyamus niger	P+	P+ young	P-		KNOTH '79 unpubl.
(Beta vulgaris	P+	P+ very seldom	P-		HOEFERT '69)
(Antirrhinum majus				P+ very seldom	DIERS '71)

Our investigations led to the conclusion that this first pollen mitosis is a very important, decisive event with regard to plastid distribution - and this is in accordance with the observations of many other cytologists. We found three possibilities for the distribution of plastids during the first pollen mitosis and further pollen development:

TYPE 1: In many species this mitosis is extremely unequal concerning the plastids. All plastids of the microspore are distributed into the cytoplasm of the vegetative cell, and the generative cell does not get any plastids. Careful electron microscopical investigations have revealed that the generative cells of many species do not contain plastids. We found this situation in *Antirrhinum majus* and in *Lycopersicon esculentum*; similar situation prevails in many other species (Table 1). This exclusion of plastids from the generative cell has of course the consequence that the two sperm cells, which are formed in the course of the second pollen mitosis from the generative cell of the pollen, do not contain any plastid either and therefore cannot transmit plastids during fertilization into the egg cell. The purely maternal inheritance of the plastids in these species is the direct consequence of exclusion of plastids from the generative cell during the first pollen mitosis (Fig. 1).

TYPE 2: A different situation exists in the second type of plastid distribution as described for *Solanum tuberosum* (by CLAUHS and GRUN 1977), and we found this for *Hyoscyamus niger* (KNOTH unpubl.). In Solanum and Hyoscyamus the first pollen mitosis is not so extremely unequal; most plastids are distributed into the vegetative cell, but also the generative cell gets some plastids. Therefore young generative cells contain some plastids.

In the course of further pollen development the plastids are, however, lost (or eliminated) from the generative cell. Thus the sperm cells no longer contain plastids. In this type,

TABLE 3. Plastid content in male gametophytes and gametes of angiosperms: Type 3.

Species	veg. cell	gen. cell	sperm cell	cross	Reference
Pelargonium zonale	P+	P+	P+	P+	KNOTH '76, '79 unpubl. KHERA '75 LOMBARDO, GEROLA '68a
Oenothera hookeri erythrosepala	P+	P+		P+	DIERS '63 MEYER, STUBBE '74
Castilleia wightii	P+	P+	P+		JENSEN, ASHTON, HECKARD '74
Linum usitatissimum	P+	P+	P+		VAZART '70
Lupinus luteus	P+	P+	P+		RUHLAND, WETZEL '24
Lupinus nootkatensis	P+	P+			LOMBARDO, GEROLA '68b
Fritillaria imperialis	P+	P+			BOPP-HASSENKAMP '60
Geranium pratense	P+	P+			KNOTH '79 unpubl.
Hippeastrum belladonna	P+	P+			LARSON '65
Impatiens glandulifera	P+	P+			RICHTER-LANDMANN '59
Lilium candidum	P+	P+			BOPP-HASSENKAMP '60
Lilium regale	P+	P+			ANDERSON '39
Lobelia erinus	P+	P+			DEXHEIMER '65

the absence of plastids in sperm cells is due to the elimination of these organelles during pollen maturation.

In this connection I want to mention one point: Almost all biological processes show occasional exceptions to the rule. It is therefore not surprising that the strict exclusion of plastids from the generative cells and sperm cells, respectively, does not always work with 100% efficiency. Most probably the types (1) and (2) are not absolutely separated; there may be situations of transition. Some plastids may in exceptional cases get into the generative cell, and even the sperm cells may very seldom get some plastids. Thus genetic experiments with *Antirrhinum majus* (which normally belongs to type 1) have demonstrated the exceptional transmission of some paternal plastids into the egg cell in 0.03% of hybrid seedlings (DIERS 1971).

TYPE 3: In striking contrast to the processes of formation of generative and sperm cells in the species of type (1) and (2) is the pollen development in the third group of species: In the genera Pelargonium and Oenothera and in some other species, list in Table 3, there is during the first pollen mitosis obviously an equal distribution of plastids to the vegetative and to the generative cell. Our investigation of the pollen development showed that the generative and the sperm cells of Pelargonium contain numerous plastids (Fig. 2). The plastids in the generative cells are smaller than those in the vegetative cells; they are frequently without starch grains, but some-

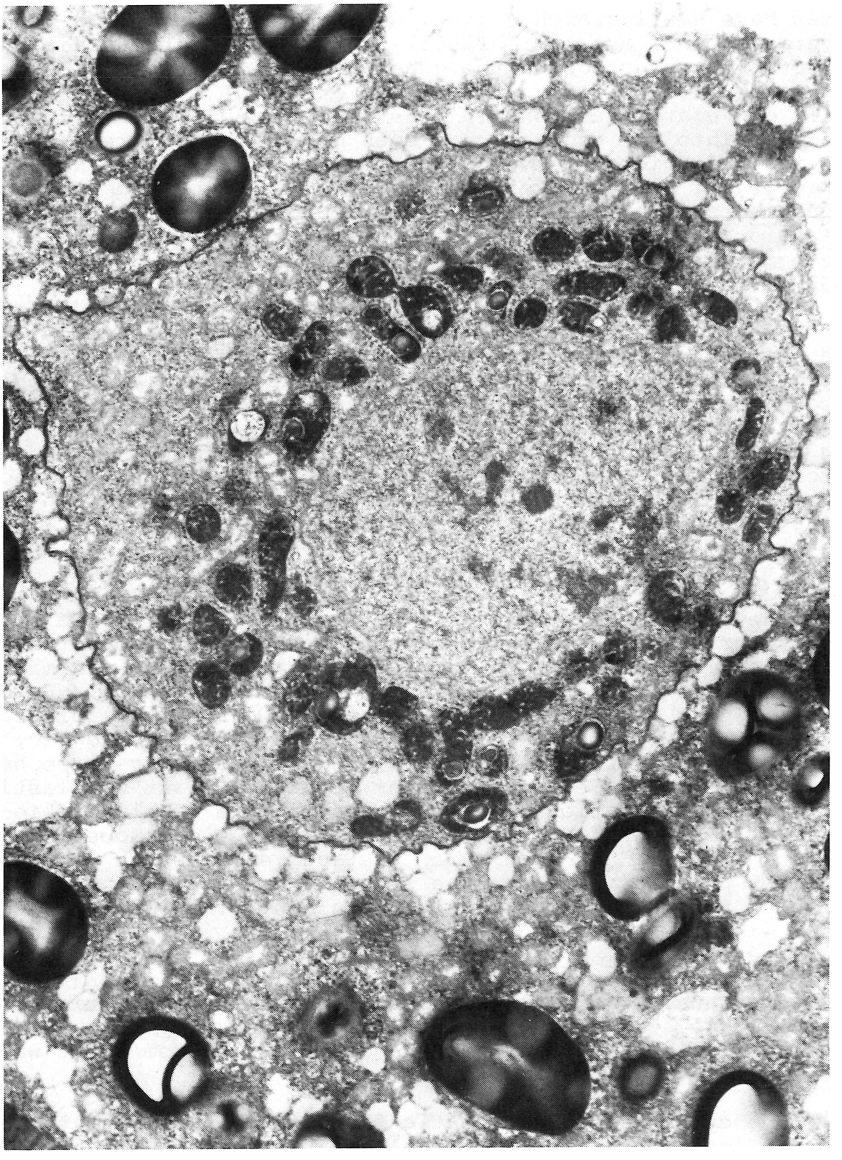

FIGURE 2. Electron micrograph of pollen cells of *Pelargonium zonale hort.* In the generative cell there are many plastids, frequently without but sometimes with small starch grains; the plastids are electron-dense, and contain accumulations of phytoferritin. The vegetative cell has many plastids with big starch grains. Fixative: Glutaraldehyde.
(Electron micrograph: R. Knoth)

times have small starch grains, whereas the plastids in the vegetative cell are amyloplasts, full of big starch grains. Moreover the plastids in the generative cells are electon-dense and accumulate phytoferritin (KNOTH et al. 1979). The sperm cells of Pelargonium contain plastids, too. Their regular transmission into the egg cell has been proved by the presence of paternal plastids in developing embryos (KHERA 1976, KNOTH 1979). Moreover, it has been well known for a long time from genetic experiments (since Erwin BAUR 1909) that in *Pelargonium zonale* transmission of paternal (green, yellow or white) plastids into the egg cell takes place regularly (cf. HAGEMANN 1964, 1965, SAGER 1972, GILLHAM 1978, KIRK and TILNEY-BASSETT 1978.

Summarizing these findings we come to the conclusion that the difference between the strictly maternal and the biparental type of plastid inheritance is based upon a relatively simple cytological mechanism: The exclusion of plastids from the generative or sperm cells during the first pollen mitosis or during pollen development in most species of angiosperms, and the absence of this exclusion mechanism in a small group of species. The physiological or biochemical means, of this exclusion remains, however, absolutely open at the moment.

It is certainly of interest in this connection that an unequal distribution of plastids in cell divisions during gametophyte development is found also in other cases. BEDNARA and RODKIEWICZ (1974) studied the development of the female gametophyte in *Epilobium palustre*. They found that plastids and mitochondria are equally distributed in the megasporocyte. But after meiosis the situation changes markedly. The haploid cell at the micropylar pole, which develops into the embryo sac, contains the majority of plastids, wheras the other three haploid cells, that degenerate later, contain almost no plastids. Comparable observations have been made in the orchid Paphiopedilum (CORTI and CECCHI 1973). In contrast, in some gymnosperms, e.g. *Cryptomeria japonica*, the active megaspore contains only few plastids, whereas the spermatogenic cells and the sperm cells of the male gametophyte contain many plastids. In accordance with these cytological findings is the fact, that genetic experiments reveal a distinct paternal bias of the plastid inheritance in these gymnosperms (OHBA et al. 1971, CAMEFORT 1966, 1975). - Thus in general, an unequal plastid distribution during cell divisions in connection with the development of male or female gametophytes or gametes is not so seldom as it was usually thought.

The cytological and genetic facts in connection with the plastid distribution during development of the male gametophyte are thus relatively clear in principle, although many details for many species have still to be worked out in the future. But there is another question which is equally important: What is the behavior of the mitochondria in the same developmental stages? Are the mitochondria in a similar way excluded from the generative cell as the plastids or are there distinct differences between these two types of plasmatic organelles? In general I must confess that our knowledge about the behavior of the mitochondria is clearly poorer than that about the plas-

tids. But I would like to report at least two sets of observations.

In the plants of the above mentioned type 3, in which there is no exclusion of the plastids from the generative and the sperm cells (Pelargonium and Oenothera), there is a lack of exclusion of the mitochondria. In other words, in plants with a biparental inheritance of the plastids there is also a biparental inheritance of the mitochondria. Pelargonium, Oenothera, Hypericum and other species with biparental plastid inheritance certainly transmit their paternal mitochondria too into the zygote. The electron micrographs regularly show mitochondria side by side with the plastids. In the other plant species there is an entire (type 1) or a prevailing exculsion (type 2) of the plastids from the generative cells. This is not so for mitochondria. The generative cells, from which the plastids had been excluded in the course of the first pollen mitosis, still contain mitochondria. However, this does not automatically mean that these mitochondria are transmitted into the egg cells. Our observations in tomato *Lycopersicon esculentum*, and in wheat *Triticum aestivum* may give a hint of the underlying processes: In young generative cells numerous mitochondria are found in electron micrographs. In older generative cells, however, the content of the cytoplasm of the cells is much reduced, and there are indications for degenerative changes in the mitochondria (e.g. the occurrence of myelin figures, which are usually considered to be a sign of degeneration). Immediately before fertilization, the sperm cells contain only a very narrow and poor cytoplasmic border. Therefore our electron microscopists have great doubts that during the process of fertilization any paternal mitochondria have a chance to be transmitted into the egg cells.

Reflecting on these findings, I have the impression that the gradual exclusion of plastids -- found in plants of type 2 (e.g. in Solanum) -- is perhaps the model for the distribution of the mitochondria in the majority of angiosperm species. A gradual exclusion or degeneration of the mitochondria has taken place in the course of the maturation of the generative cell and the sperm cells, which by degrees leads to the loss of paternal mitochondria before the onset of fertilization. However, I should emphasize, that this explanation rests upon a very small number of observations, and I was surprised at the lack of information in the literature concerning this question. At present our research group is studying this problem intensively and we hope to find out many more details and facts concerning this issue which is important both from the theoretical and from the practical point of view, since in experiments of hybridization of different species or genera it seems to be very important to know exactly from what parent the plasmatic organelles, plastids and mitochondria, originate.

EXPERIMENTAL INDUCTION OF PLASTID MUTATIONS IN HIGHER PLANTS WITH THE AID OF CHEMICAL MUTAGENS

In 1927/28 H. J. Muller and L. J. Stadler working in the United States proved that X-rays are powerful for inducing mu-

tations in Drosophila as well as in higher plants, and in 1929-30 H. Stubbe and N. W. Timoféeff-Ressovsky confirmed these results in Europe for the same groups of organisms. Very soon afterwards the experimental induction of mutations by ionizing radiation, by UV, and later by a great variety of chemical compounds became a widely used method in many fields of genetics of eukaryotic and prokaryotic organisms. In this respect, geneticists working on plstid genetics in higher plants have had, however, great problems in the past. They tried for a long time to experimentally induce plastome mutations in higher plants by physical and chemical agents in the same way as the induction of nuclear gene mutations in all groups of genetic objects and as the induction of mitochondrial mutations in yeast and of plastid mutations in Chlamydomonas and Euglena. However, for many years experimental attempts failed or gave unsatisfactory or contradictory results.

But now there are a number of reports about the successful induction of plastome mutations by means of nitroso-methyl-urea (NMU) in Helianthus and Saintpaulia. Since 1969 the research group of BELETSKI in Rostow/USSR has been reporting about the induction of plastome mutations in the sunflower *Helianthus annuus*. In the meantime we have been able to confirm this result; we found that the sunflower is particularly sensitive to the mutagenic action of NMU. In addition three research groups in our country, namely the groups of HENTRICH and BEGER (1974), POHLHEIM (1974) and JUNGNICKEL (1977) have been working with the small ornamental plant *Saintpaulia ionantha* and have induced with NMU many variegated plants which were due to plastid mutations. The only disadvantage of these interesting studies is the fact that both species, the sunflower and Saintpaulia, have so far played no role in plastid or in nuclear genetics.

This led us to perform mutation experiments with two standard objects of genetics: with snapdragon, *Antirrhinum majus*, and the tomato, *Lycopersicon esculentum*. Another aspect of our work was that we not only used the nitroso-compound N-nitroso-N-methyl-urea (NMU) but also the compound N-nitroso-N-ethyl-urea (NEU) and could thus compare these compounds, both of which are strong mutagens and effective carcinogens (HAGEMANN 1976). These experiments in our lab were done in cooperation with Mrs. Grimmer, Lieberwirth and Scholze. In detail, we studied the following aspects.

(1) EFFECTIVENESS OF TREATMENT FOR OBTAINING VARIEGATED PLANTS. We treated seeds of both Antirrhinum and Lycopersicon with solutions of the mutagens and tested -- after presoaking the seeds in water (6 hours for Antirrhinum and 16 hours for tomato) -- various concentrations of the mutagens between 1 and 31 millimole per liter. In these experiments we found an interesting difference between tomato and Antirrhinum. In tomato almost all seeds -- treated with NMU concentrations between 1 and 20 millimole/l -- yielded some surviving plants. The plantlets showed typical characteristics of the action of the mutagent in the seedling stage. Afterwards the seedlings grew into surviving plants. The results of two sets of experiments show that the increase in variegated plants is correlated with the in-

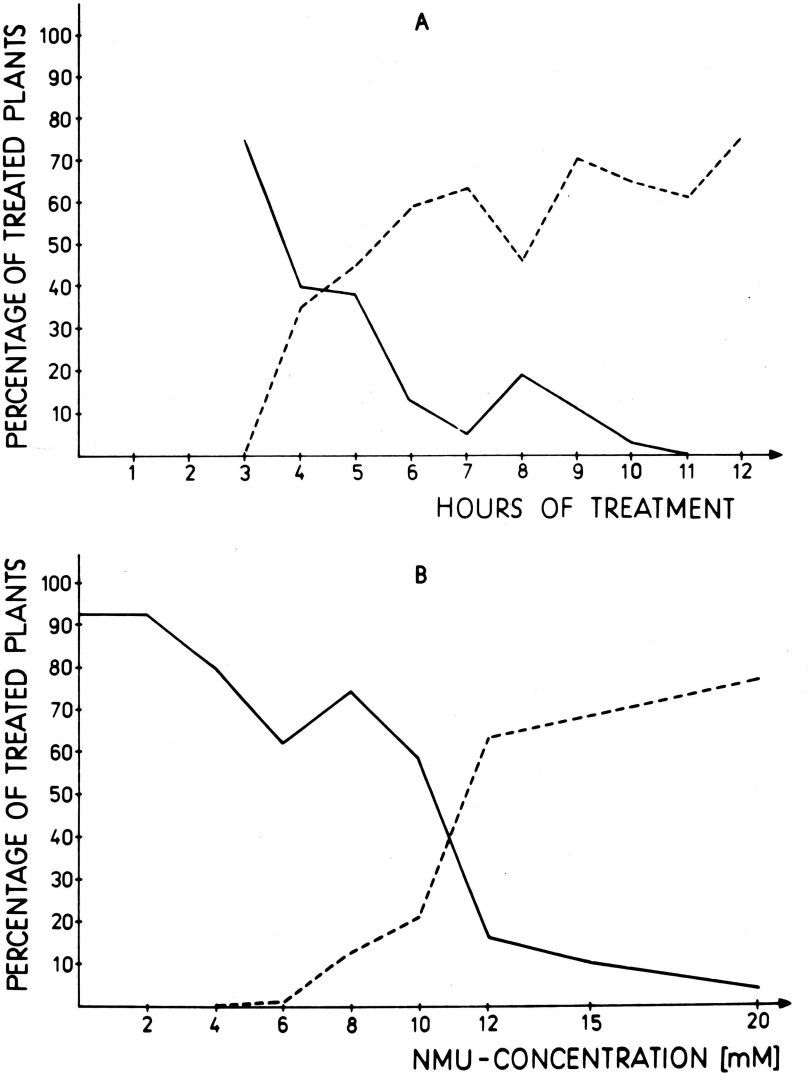

FIGURE 3. Correlation between survival (——) after treatment with NMU and the increase in variegated plants (----) of the tomato, *Lycopersicon esculentum*.
A Treatment of seeds with a 5 mM solution of NMU for varying time
B Treatment of seeds for 3 hours with varying concentrations of NMU
(Experiments of U. Grimmer)

crease in the action of the mutagen: The longer the duration of treatment with a 5 millimolar solution of NMU, the higher the percentage of variegated plants among all developing plants (Fig. 3A). When the plants were treated for 3 hours with different concentrations of NMU, it can be seen that the number of variegated plants increases with an increase in the concentration of the mutagen (Fig. 3B). In *Antirrhinum majus* the number of surviving seedlings greatly decreases with increasing concentrations of the mutagenic solution. With increasing concentrations of the mutagenic solution an increasing proportion of the surviving seedlings variegated (Table 4). From the practical aspect of inducing variegated plants in Antirrhinum, we found that a treatment of seeds with 10 to 15 millimolar NMU solutions for 2 or 3 hours resulted in the highest number of variegated plants.

(2) COMPARISON OF NMU AND NEU. In *Antirrhinum majus* we systematically compared the action of nitroso-methyl-urea and nitroso-ethyl-urea and found an approximate twofold dose of NEU as efficient as NMU. In mutation experiments with durum wheat (DESAU and BHATIA 1975) NMU was found to be more toxic than NEU, whereas the mutagenic efficiency of NMU was considerably higher - which is in full accordance with our results.

(3) PROPORTION OF PLASTOME MUTANTS AMONG THE INDUCED MUTATIONS. We are fully aware of the fact that NMU and NEU are very efficient mutagens and carcinogens and that they induce not only plastome mutations, but also many nuclear gene and chromosome mutations. The recessive gene mutations are detected only in M_2. Therefore, we focused our attention on variegated M_1 plants. Part of them are certainly dominant nuclear mutations or chromosome aberrations, but part of them are plastome mutations. The plastome mutations among the M_1 plants with chlorophyll deficiencies were found by using 3 criteria: (a) the fine pattern of variegation in color which is typical for sorting out of genetically different types of plastids, (b) the cytological demonstration (by light and electron microscopy) in these plants of mixed cells containing in the same cell two (or even more than two) different types of plastids (normal green and white or yellow or light green) mutant plastids and (c) the proof of the non-Mendelian, uniparental type of inheritance of these characters in Antirrhinum and Lycopersicon.

One of the main objectives in inducing plastome mutants with chemical mutagens was to obtain new types of plastome mutants for our cytological and biochemical studies. During the past 8 years our research group made great efforts to characterize several plastome mutants of Antirrhinum, Pelargonium and barley. These mutants have been partly spontaneous and partly gene-induced plastome mutants. The effective use of nitroso-methyl-urea and nitroso-ethyl-urea makes it now possible to enlarge the number of plastome mutants available for such studies.

BIOCHEMICAL AND MOLECULAR ANALYSIS OF PLASTOME MUTANTS IN HIGHER PLANTS

TABLE 4. Induction of variegated plants of *Antirrhinum majus* by treatment with N-nitroso-N-methyl-urea (NMU) and N-nitroso-N-ethyl-urea (NEU). From HAGEMANN (1976).

Mutagen treatment	Concentration (mM)	Total number of seedlings	Number of variegated seedlings	Number and percentage of variegated seedlings
Presoaking 6 hours followed by 2 hours NMU[1]	4	150	4	2.7
	5	112	4	3.6
	6	128	3	2.3
	7	149	8	5.4
	8	155	8	5.2
	9	119	10	8.4
	10	96	21	21.9
	11	28	14	50.0
	12	39	23	59.0
	13	27	13	48.1
	14	24	18	75.0
Presoaking 6 hours followed by 2, 5 hours NMU[2]	4	174	1	0.6
	5	103	0	0
	6	134	7	5.2
	7	138	10	7.2
	8	81	7	8.6
	9	50	11	22.0
	10	120	24	20.0
	11	108	34	31.5
	12	86	31	36.0
	13	82	24	29.3
	14	91	17	18.7
	15	68	28	41.2
	16	42	7	16.7
	17	18	17	94.4
Presoaking 6 hours followed by 3 hours NEU[2]	4	153	1	0.7
	7	190	2	1.1
	10	127	4	3.2
	13	156	14	9.0
	16	99	10	10.1
	19	117	21	18.0
	22	109	21	19.3
	25	78	14	18.0
	28	32	8	25.0
	31	24	10	41.7

[1] Experiment of M. Scholze
[2] Experiments of F. Lieberwirth

Biochemical analysis of plastome mutants in Antirrhinum, Pelargonium and Hordeum was performed by the following members of our research group: Drs. Borner, Herrmann, Knoth, Schumann and Grimmer. We concentrated our efforts especially on plastome mutants. They are caused by mutations in the plastid DNA itself. In studying the structural, functional and biochemical changes in such plastome mutants we try to define plastome-controlled metabolic reactions, to get information about the coding functions of the plastid DNA and to determine the synthetic capacity of the plastid as an organelle. In the course of our studies we found two different groups of plastome mutants.

PLASTOME MUTANTS WITH SPECIFIC DEFECTS IN PHOTOSYSTEMS

These mutants have a normally working protein-synthesizing system in their plastids. But the mutation in the plastid DNA causes defects in the synthesis of particular proteins connected with the photosystems. We have analyzed three mutants of this type in detail. Two mutants proved to have a defect in photosystem I: the mutant *en:alba-1* of *Antirrhinum majus* and the mutant plastids of the Pelargonium variety "Mrs. Pollock". One mutant showed a defect in photosystem II: the mutant *en:viridis-1* of *Antirrhinum majus*. Various methods have been used to characterize photosynthetic light reactions; biophysical methods such as measurement of electron spin resonance (ESR), of delayed light emission (DLE) and of increased in vivo fluorescence as a signal for a deficiency of photosystem I; polyacrylamide gel electrophoresis of SDS-solubilized chlorophyll-protein-complexes and thylakoid proteins (Table 5). The analysis of these three mutants revealed that the mutant plastids of *en:alba-1* (Antirrhinum) and "Mrs. Pollock" (Pelargonium) have a specific deficiency in photosystem I; they also lack the protein(s) of the chlorophyll protein complex I, associated with photosystem I (Fig. 4, 5). - On the other hand, the plastids of *en:viridis-1* (Antirrhinum) have a deficiency of photosystem II; the lamellar protein pattern demonstrates that the protein band 7 is no longer detectable and the protein bands 8 - 12 are markedly decreased (Fig. 4). In this connection it is interesting to note that the reaction center of photosystem II is associated with a lamellar protein of a molecular weight of 40000 to 45000 which is to be expected in our gels in the range of bands 7 to 10 (HERRMANN 1971, 1972: HERRMANN et al. 1974, 1976; HERRMANN and HAGEMANN 1972; MACHOLD and HOYER-HANSEN 1976). These three plastom mutants carry changes in their plastid DNA (while the genetic information of their nucleus is intact). These mutations lead to a loss of particular bands of thylakoid proteins, which are associated with photosystem I or photosystem II, or a marked decrease in band intensity, thus causing specific defects in the electron transport chain of the photosystem. We conclude from these findings that the plastid DNA controls the formation of particular components of the tylakoid proteins. As far as I can see, our laboratory was the first one to report on the analysis of plastome mutants with specific defects in a photosystem and the associated loss of specific lamellar proteins. Meanwhile several labs have reported on similar findings for other objects, especially for Scenedesmus and Chlamydomonas (cf. the references in HAGEMANN 1979). These findings

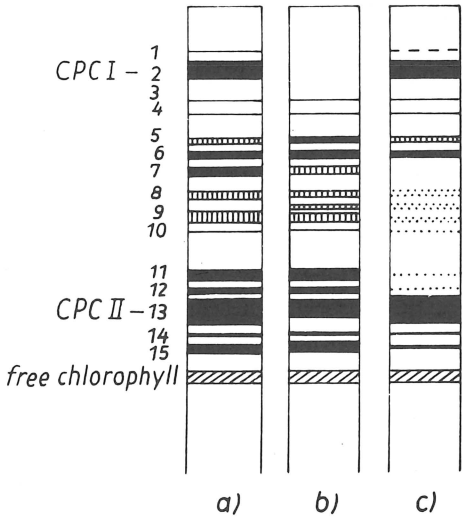

FIGURE 4. Electrophoretic pattern of plastid lamellar proteins of *Antirrhinum majus*.
a) wild type
b) plastome mutant *en:alba-1* with defect in photosystem I
c) plastome mutant *en:viridis-1* with defect in photosystem II
CPC: chlorophyll-protein-complex

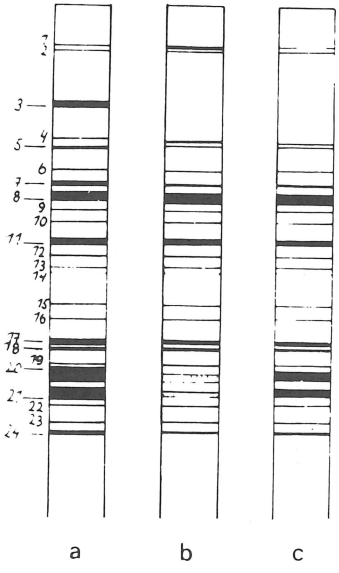

FIGURE 5. Electrophoretic pattern of plastid lamellar proteins of *Pelargonium zonale*.
a) wild type plastids
b) plastids of "Mrs. Pollock" (plastome mutant) grown under intense light conditions
c) plastids of "Mrs. Pollock" (plastome mutant) grown under dim light
Band No. 3 corresponds to CPCI
(From HERRMANN et al. 1976)

TABLE 5. Analysis of plastome mutants of *Antirrhinum* and *Pelargonium* with defects in photosystem I or photosytem II

	Antirrhinum majus *en:viridis-1*	*en:alba-1*	*Pelargonium zonale* "Mrs. Pollock"
chlorophyll content in dim light	37 %	38 %	41 %
photosynthesis	no	no	no
plastid ribosomes	yes	yes	yes
RuBP carboxylase	yes	yes	yes
activity of dark reactions (Calvin cycle)	yes	yes	? (inhibitors)
active photosystem I	yes	no	no
DCPIP·H_2+ ascorbate anthraquinone	yes	no	?
ESR signal	yes		no
increased in vivo fluorescence		yes	yes
active photosystem II	no	yes	yes
DCPIP, ferricyanide	no	yes	?
DLE	almost no		yes
lacking lamellar protein bands	No. 7 (No.8-12 reduced)	CPC I (1,2)	CPC I (3)

clearly demonstrate the important role of the plastid DNA in controlling the biogenesis of the chloroplast.

PLASTOME MUTANTS WITH PLASTID RIBOSOME DEFICIENCIES

In recent years our research group has found several plastome mutants of *Pelargonium zonale* and *Hordeum vulgare* which have no (or almost no) plastid ribosomes. These are the white plastids of the white-margined Pelargonium varieties "Mrs. Parker", "Freak of Nature", "Flower of Spring", "Gnom" and "Madame Salleron" and the white plastids in white or striped leaves of the barley mutant lines 'albostrians' and 'Saskatoon'. The absence of plastid ribosomes has been demonstrated both by biochemical and electron microscopical investigations. Whereas normal green leaves contain four fractions of high-molecular-weight ribosomal RNAs, 25S and 18S of cytoplasmic ribosomes and 23S and 16S of plastid ribosomes (Fig. 6), the white mutant leaves only possess 25S and 18S rRNAs (Fig. 6); the 23S and 16S rRNAs of the plastid ribosomes are completely or almost entirely ab-

sent (BÖRNER et al. 1972, 1973). In accordance with these findings, electron micrographs demonstrate the absence of plastid ribosomes in differentiated white cells (Fig. 7) and the presence of cytoplasmic ribosomes in normal amounts in the same cells. (Mutant barley plastids do not contain plastid ribosomes in any developmental stage. In Pelargonium, in very young proplastids of young leaves we could find in very rare instances a few plastid ribosomes.) These mutants have no (or almost no) plastid ribosomes. Hence their plastidal protein-synthesizing system is entirely blocked, whereas the cytoplasmic protein-synthesizing system is not impaired. These mutants are thus a very suitable tool for elucidating the synthetic capacity of the plastidal protein synthesizing system and -- on the other hand -- of the contribution of cytoplasmic protein synthesis to the formation and assembly of the photosynthetic machinery of the plastid. All proteins, found in such ribosome-

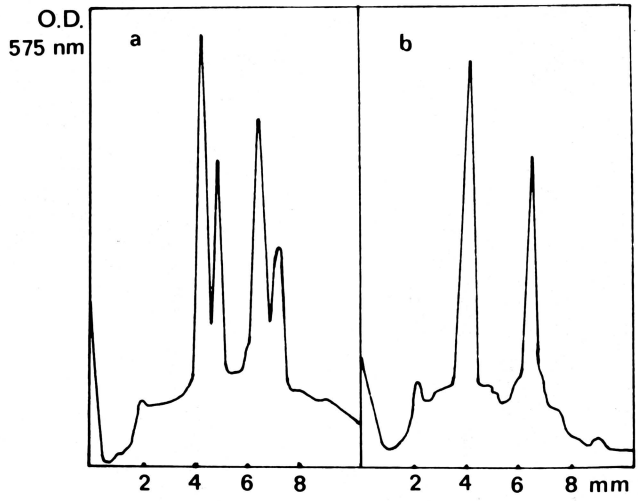

FIGURE 6: Separation of high molecular-weight rRNA in polyacrylamide gels. a: RNA of green leaves, b: RNA of white light-grown leaves (from BÖRNER et al. 1976)

deficient plastids, in spite of their deficiency, have obviously been synthesized on cytoplasmic ribosomes and subsequently transported into the plastids.

PLASTID COMPONENTS SYNTHESIZED ON CYTOPLASMIC RIBOSOMES

Our investigations (cf. HAGEMANN and BÖRNER (1978) demonstrated the presence of the following plastid components in the plastid-ribosome-deficient mutants:

(1) THE DNA POLYMERASE OF THE PLASTIDS. White ribosome-deficient mutant plastids of Pelargonium and barley contain plastid DNA which is replicated, as shown by autoradiography (KNOTH et al. 1974). The DNA polymerase performing this replication is coded by nuclear DNA, it is synthesized on cyto-

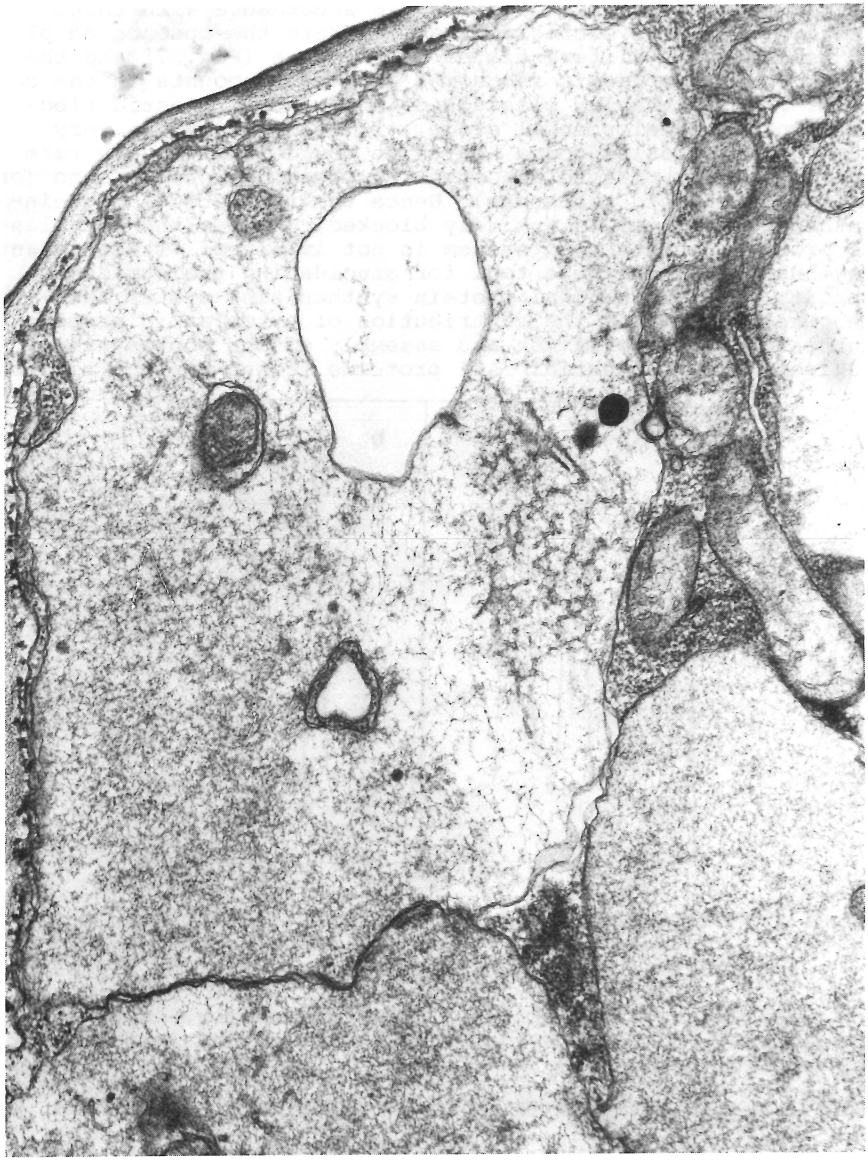

FIGURE 7: Electron micrograph of a cell of *Pelargonium zonale hort.* Plastome mutant "Mrs. Parker", with ribosome-defieient plastids. The plastids do not contain any ribosomes. But cytoplasmic ribosomes are normally present in the surrounding cytoplasm and within invaginations. Fixative: Glutaraldehyde. (Electron micrograph: R. Knoth)

plasmic ribosomes and then transported into the deficient plastids.

(2) COMPONENTS OF THE PLASTIDAL ENVELOPE. Ribosome-deficient plastids have a double-layered envelope like normal chloroplasts (BÖRNER et al. 1972, KNOTH and HAGEMANN 1977). Possibly some minor components may be missing in this envelope, but the major components are obviously synthesized in the cytoplasm.

(3) COMPONENTS OF THE PROLAMELLAR BODY. Ribosome-deficient plastids in dark-grown leaves of Pelargonium and barley mutants contain prolamellar bodies (KNOTH and HAGEMANN 1977). These components are also formed in the cytoplasm and then transferred into the plastids.

(4) SOME CALVIN CYCLE ENZYMES. The enzymes phosphoribulokinase and NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase can be found in mutant white barley plastids, although the level of activity is only about 5% compared with the control. They are also synthesized on cytoplasmic ribosomes (BRADBEER and BÖRNER 1978).

(5) ENZYMES FOR CHLOROPHYLL AND CAROTENOID SYNTHESIS. Young leaves of the Pelargonium mutants grown under dim light do contain chlorophyll (1.5%) and carotenoids (10% of the control) (BÖRNER unpubl.). Their presence points to cytoplasmically formed enzymes for their synthesis.

(6) STARCH-FORMING ENZYMES. Ribosome-deficient plastids are able to form starch. The appropriate enzymes are synthesized on cytoplasmic ribosomes.

(7) SYSTEM FOR TRANSPORT INTO THE PLASTIDS. From the results just reported, it can be concluded that the transport system which enables the transport of cytoplasmically translated proteins and other compounds into the plastid is intact in the ribosome-deficient plastids. The protein components of this transport system are thus also synthesized in the cytoplasm.

PLASTID COMPONENTS SYNTHESIZED ON PLASTID RIBOSOMES

Proteins which are normally synthesized within the plastids cannot be formed in ribosome-deficient plastids. Therefore the analysis of these mutants also give information about this group of plastid components. But one has to be critical. If we cannot find a particular protein in a plastid-ribosome-deficient mutant, we are not allowed to uncritically state that in wild type plants this protein is synthesized within the plastid. It may also be possible that a protein is formed in the cytoplasm, but as a result of a signal from the plastid. If this signal does not come out of the organelle - because the organellar protein synthesis is blocked - then this protein is not synthesized in the cytoplasm. But taking into account also the results of studies with specific inhibitors (cf. BÖRNER 1973), experiments with isolated chloroplasts (cf. ELLIS 1977),

studies of in vitro synthesis with the aid of plastid mRNA (HARTLEY et al. 1975) and the results of experiments with linked transcription-translation systems using plastid DNA fragments (cf. BOGORAD et al. 1978), we can draw conclusions from our studies with plastid ribosome-deficient mutants about the synthetic capacity of the plastids. Presupposing this, we concluded that the following four protein complexes are missing in plastid-ribosome-deficient mutants, because particular polypeptides of them are normally synthesized on plastid ribosomes (HAGEMANN and BÖRNER 1978):

(1) RIBULOSE-1,5-BIPHOSPHATE CARBOXYLASE (RuBPCase). The small subunit of this enzyme complex is encoded in the nuclear DNA, synthesized on cytoplasmic ribosomes and then transported into the plastid. In contrast, the large subunit is encoded in the plastid DNA and synthesized on plastid ribosomes (CHAN and WILDMAN 1972, UCHIMIYA et al. 1977, ELLIS 1977). Immunological studies and polyacrylamide gel electrophoresis of SDS-treated soluble proteins of our barley mutants failed to reveal the large and the small subunits of RuBPCase (BÖRNER et al. 1973, 1974, 1976).

(2) PLASTIDAL COUPLING FACTOR CF_1 (PLASTIDAL ATPase). CF_1 consists of five subunits. Protein synthesis in isolated chloroplasts suggested the synthesis of the two largest and possibly of the smallest subunit within the plastid (MENDIOLA-MORGENTHALER et al. 1976, ELLIS 1977, KWANYUEN and WILDMAN 1978). Immunological, gel electrophoretic and electron microscopical studies failed to find the plastidal Coupling factor CF_1 in our barley mutant 'albostrians'.

(3) and (4) PROTEIN COMPLEXES ASSOCIATED WITH PHOTOSYSTEM I AND II. Plastid ribosome-deficient mutants of barley and Pelargonium do not exhibit the normal signals of electron spin resonance (ESR) and delayed light emission (DLE) (MATORIN and BÖRNER unpubl.). This indicates defects both in photosystem I (tested with ESR) and in photosystem II (tested with DLE) (Table 5).

The analysis of plastome mutants is still far from being completed, and I was also not able to deal with all aspects of these studies in this lecture (cf. HAGEMANN and BÖRNER 1978, HAGEMANN 1979). But we can already state that such mutants are interesting and stimulating means for characterizing the synthetic capacity of the plastids and the contribution of the protein-synthesizing system of the cytoplasm to the biogenesis of the chloroplasts.

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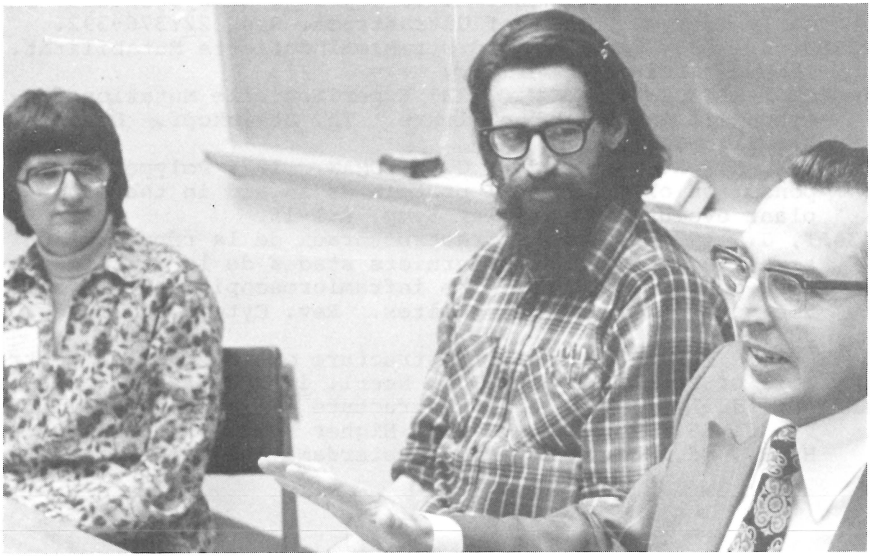
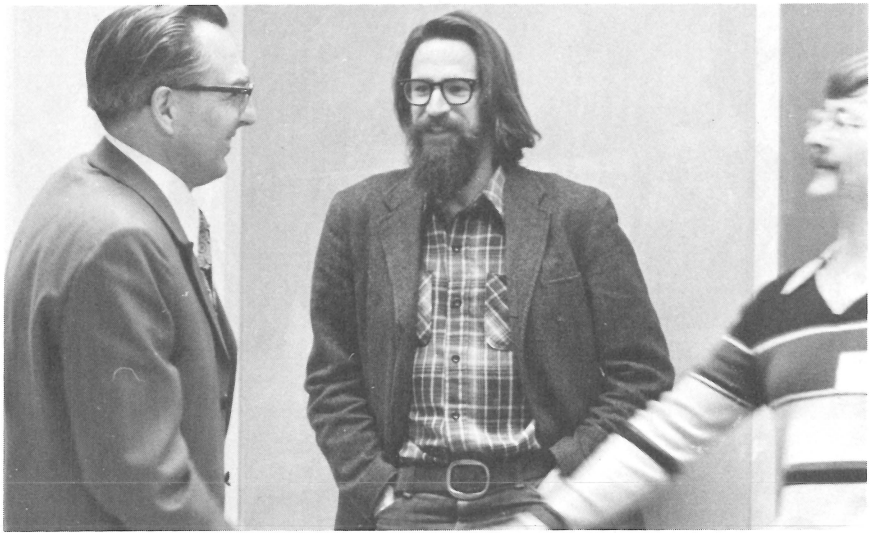
Dr. Hagemann (right) in the discussion group with Dr. George Smith.



Left to right: Drs. Barker, Smith and Hagemann.



Drs. Hagemann, Smith and Merlo at the Symposium.